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THE BACILLUS OF FOOT-ROT IN SHEEP.

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The prevalence of contagious foot-rot in sheep in Ohio, the fact that Ohio is a wool-producing state, that the animal loss to the state is to be estimated by thousands of dollars, that the specific nature of foot-rot has never been investigated, that remedies consist in the use of mineral acids and alkalies that are highly caustic in their effects, — all these combined, induce me to consider this disease a fit subject for investigation with a view to a more rational system of treatment.

Accordingly, January 10, 1887, the investigation was begun in the following manner: A piece of white silk thread known as surgeon's silk, was infected with the discharge which issued from between the toes of a sheep having foot-rot, in Jefferson county, Ohio. The thread was then carefully dried and sent in a letter to Dr. H. J. Detmers, Columbus, Ohio, thus exposing it to the temperature of January. On February 13, 1887, small pieces of the thread, say one third inch long, were put into sterilized gelatin culture tubes which were then allowed to rest obliquely.

The gelatin culture tubes were prepared in the following manner: Pieces of meat, of young animal preferred, were boiled down to a jelly at a temperature of about 165° F. for six to eight days, then filtered in a water-oven into sterilized test-tubes, say two inches deep, and the tubes closed with a plug of absorbent cotton which had been thoroughly disinfected. The tubes were heated two hours daily for five days in the water-oven at a temperature of 165° F. and allowed to cool at an angle of 45°.

One of the tubes seemed to be contaminated with a fungus and was discontinued. The other presented a delicate white growth near the thread and on the surface of the gelatin which showed two things. First, that the bacteria are incidental parasites by living on the thread and, secondly, that they are aërobic, that is, that they need the oxygen of the air for their development; this explains why they remain as a local source of irritation and do not enter the blood stream and produce general disease.

On February 27, 1887, another culture tube was inocculated from tube No. 1, in the following manner: The culture tube was held in the left hand in a horizontal position, the plug of cotton removed with the right hand and held between the fingers of the left hand so that nothing could fall on it. Then a platinum wire, that had been disinfected by heating in the flame of a spirit lamp and allowed to cool, was introduced, and a small quantity of the gelatin adhered to the wire, then the cotton was replaced and the material was introduced into the second culture tube while being held as before. Cover glass preparations were then made in the following manner: A very small drop of distilled water was put on a clean cover-glass and with a platinum wire, having been first heated in a spirit lamp, a minute portion of the gelatin culture No. 1 was taken from the tube (held almost horizontally) and mixed with the drop of water on the cover glass, and so distributed as to make a thin, even spread. Then with a pincette the cover-glasses were placed under a bell glass and allowed to dry. When dry they were taken with the pincette and passed quickly through the flame of a spirit lamp three times, bacteria side up, then dropped into a watch glass containing a solution of Bismark brown or vesuvine that had been previously filtered.

The covers were allowed to float thus in the staining fluid from five to seven hours according to the intensity of the stain desired. When sufficiently stained they were taken with a pincette and washed, either passed quickly through a beaker of water by a to-and-fro motion, or washed with a wash bottle until the water came away clear, and again put under a bell glass and allowed to dry. When dry they were mounted in Canada balsam.

Several cultures were made at intervals of a week or ten days according to the weather, and cover-glass preparations were made in the manner heretofore described. On March 17, 1887, a merino ram was procured for experimental purposes. This ram was from a flock that never had the disease, nor was there ever any of the disease on the farm. The feet were inspected by Dr. Detmers, myself, and others at the time of his arrival and all were perfectly normal. It was soon decided to inoculate one or more of the feet near the opening of the inter-ungulate gland, which was done in the right fore foot as follows: The foot was thoroughly washed by myself with warm water and soap, then with an aqueous solution of corrosive sublimate, 1 to 1000, then with alcohol and the latter allowed to evaporate.

By means of a small inoculating needle, disinfected in the flame of the spirit lamp, a small portion of gelatin culture, No. 4, was introduced just under the integument near the duct of the inter-ungulate gland. The ram was then kept in a clean, board-floor stall of the veterinary hospital, which never had been occupied by any sheep, and none had ever been in close proximity to it. Daily observations were made by myself and others; at first a little irritation and redness were present, probably due to the inoculating needle. In five days increased heat was quite perceptible, but it gradually subsided and nothing very abnormal could be observed except some fluid exhudation, or a little moisture. This condition of affairs continued, as daily observations were made by myself and others, until about the first of April. From that date appeared a rather foetid, whitish or yellowish discharge, which gradually increased until April 26, when it was pronounced genuine foot-rot by Drs. Townshend, Detmers, and all who saw it and were familiar with the disease. This condition of affairs continued only in a more aggravated form. The other feet remained normal, probably due to the fact that the ram was kept constantly in a dry place so that the exhudation dried before it came into a suitable nidus for its development. On April 29, a sterilized gelatin culture tube was inoculated with a portion of the discharge from the ram, with the platinum wire previously heated, and cultures made at intervals of a week or ten days. Numerous cover-glass preparations have been made of each of the cultures in the method already described.

Under the microscope with $\frac{1}{6}$ inch dry objective, Abbè condenser, Bulloch's biological stand, No. 9 eye-piece, and lamp-light, unstained cover-glass preparations in water showed numerous bacteria in motion, therefore they are motile.

At a superficial examination, especially in over-stained preparations, the bacteria might be mistaken for "oval micrococci" (Huppè, *Methods of Bacteriological Investigation*).

These presented themselves as single, short, rather angular rods, only a trifle longer than thick. They measured about 1.μ in length and about 0.7μ in thickness. There were double forms, two short rods joined by their ends, and also chains, of various lengths. These are no doubt true Bacilli. If we accept "oval Micrococci," and according to Huppè we must, these are somewhat different, having quadrangular parallel sides, and perhaps a little beveled corners. The joints are not the same length in different media. In different samples of gelatin a difference can be observed which could not happen if they were "oval Microcci." They are also different in new and old cultures, those of a fresh culture being larger and much more resembling a typical Bacillus.

Under a $\frac{1}{15}$ homogeneous immersion objective of Tolles, N. A. 1.30, Abbè condenser, Beck's No. 1 eye-piece, tube length 238 mm., and lamp light, making an amplification of 845 diameters, the long chains showed to be bacilli without a doubt; a dark spot was also visible in each which was regarded as a nucleus. These nuclei were not to be seen with the dry $\frac{1}{6}$ objective and lamp light.

The size of the Bacilli were about $1.\mu$ in length and about 0.7μ in width, which was determined in the following manner: A Zeiss glass rule, accurately divided into half millimeters, was held on the negative over the image of the Bacilli and they were found to be fully 1 mm. long and 0.7 mm. wide, and as the amplification of the negative was 1040, therefore the above results.

The vitality of the virus is a question of utmost importance to be determined. It will be seen that the virus survived the exposure of January weather from the time the thread was infected, until the gelatine tubes were inoculated in a dried condition, and kept in an envelope in a living room that was comfortable in the day time and cold at night. How much more exposure it would stand has not been determined, but the above was fully demonstrated. The cultures were discontinued in June, 1887, on account of the warm weather, but will be started again in the fall with a view to treatment, methods of prevention, etc.